HUNTINGTON MEDICAL RESEARCH INSTITUTES

NEUROLOGICAL RESEARCH LABORATORY

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Pasadena, California 91105

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"SAFE AND EFFECTIVE STIMULATION OF NEURAL TISSUE"

William F. Agnew, Ph.D.

Douglas B. McCreery, Ph.D.

Ted G.H. Yuen, Ph.D.

Randy R. Carter, Ph.D.

Leo A. Bullara, B.A.

This QPR is being sent to you before it has been reviewed by the staff of the Neural Prosthesis Program

ABSTRACT

The March 31, 1995, QPR reported results from a total of 19 unpulsed arrays of microelectrodes implanted in the cerebral cortex of 11 cats. This report present the histologic results from 12 cats following implantation of unpulsed iridium microelectrodes having one of 4 tip configurations and implanted either manually or with a vacuum-operated holder mounted to the stereotaxic head frame. Forty-seven electrodes were implanted in the cerebral cortex of the 12 cats. Because of bent electrode tips, results from two electrodes with 1 μm diameter rounded tips were excluded. Virtually all one-day implants were associated with small-to-large interstitial hemorrhages. The 14 and 28-day implants were not hemorrhagic but showed gliosis and vascular changes (hyperplasia and/or hypertrophy) close to the electrodes.

INTRODUCTION

Boast et al (1976) described extensive vascular disruption following the insertion of penetrating electrodes into mouse brain (2 twisted wire electrodes totaling 350 μm in diameter). They also commented on the high incidence of hemorrhages associated with the penetrating electrodes and concluded that, though their electrodes were somewhat large, the large number and distribution of cortical blood vessels was primarily responsible for the numerous hemorrhages.

The present report is a continuation of previous studies to determine the characteristics and extent of trauma associated with the penetration into the cerebral cortex of microelectrodes with various electrode tip configurations.

METHODS

I. **Electrodes.** All iridium microelectrodes were 1.5 mm in length, 50 μm in diameter and coated with 2 layers of Epoxylite. The tops of the microelectrode shafts were embedded in an epoxy matrix which was 2.5 mm in diameter and 0.5 mm thick (Fig. 1). Four types of tip configuration were used (Figs. 2 to 4) and the insulation was removed from the distal portion of the shaft to expose 500 μm^2 of metal.

II. **Electrode Implantation.** Adult cats were anesthetized with Halothane and nitrous oxide. Using aseptic surgical technique, a 1 x 1 cm craniectomy was performed in the parietal region

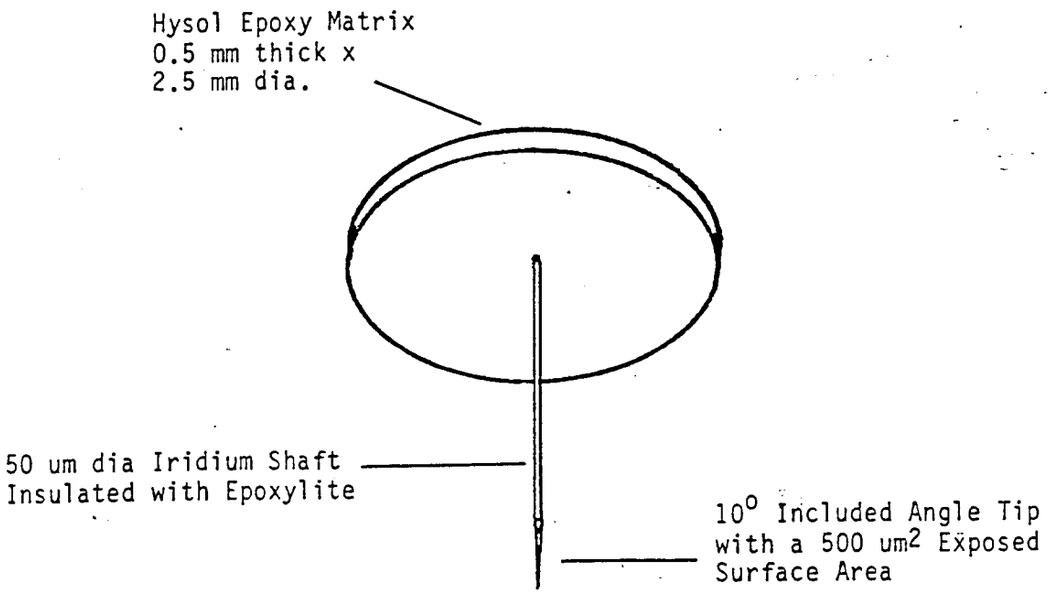


Fig. 1: Intracortical Iridium Electrodes

Iridium shafts with either 1.0, 3.0 or 6.0 um dia tips or ellipsoidal flat area tips. Exposed surface areas for all tip configurations are 500 um^2.

Figs. 2 & 3. Fifty μm diameter microelectrode shafts having rounded tip with diameters of 1 and 6 μm, respectively. The distal end of each microelectrode, including those with 3 μm-diameter rounded tips, has an exposed, insulation-free area of 500 μm². Each division of graticule = 14.5 μm.

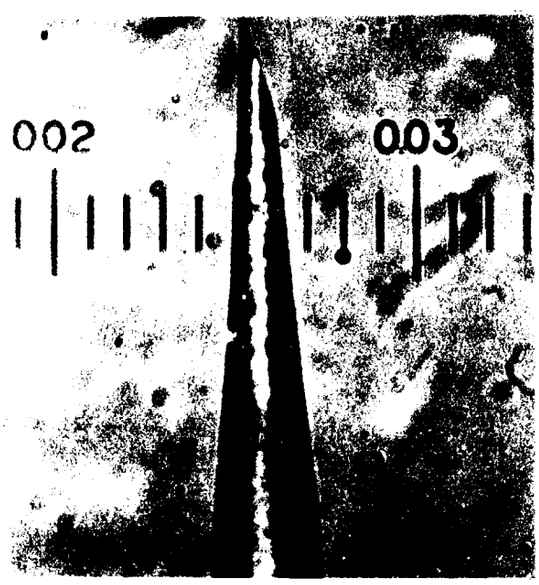


Fig. 4. Side view of an iridium intracortical electrode with a beveled, ellipsoidal facet at the tip. The activated (uninsulated) surface area of the facet measures 500 μm². Each division of graticule = 14.5 μm.

of each hemisphere. The dura was reflected back as a U-shaped flap to expose the gyrus suprasylvius. Each gyrus suprasylvius was implanted with two individual electrodes whose tips were identical to those of their neighbors but of a different design from those on the corresponding, contralateral gyrus. The electrodes were inserted either manually (using specifically designed tweezers having cup-shaped tips) or with the aid of a stereotaxic carrier to which was attached a vacuum-assisted electrode holder mounted on a microdrive. A special effort was made to avoid penetrating blood vessels on the surface of the cortex. The microelectrodes were inserted quite slowly (approximately 1 mm/sec). The dura was reapproximated and sutured with continuous sutures. Bacitracin was applied to the wounds and the wounds were closed in layers.

A total of 10 electrodes with each of the 4 tip configurations were implanted (conical tips tapering to 1, 3, or 6 μm diameter rounded points, and those with conical tips terminating in an elipsoidal facette). Six electrodes of each type were implanted manually and 4 implantations were effected by using the vacuum introducer. The implant duration was one day. In addition, one animal was implanted for 14 days, with microelectrodes with 6 μm-diameter rounded tips, and one animal was implanted with 1 and 3 μm-diameter rounded tip electrodes for 28 days (Table 1). At the end of the implant period the animals were deeply anesthetized and sacrificed by transcardiac perfusion of Karnovsky's fixative followed by storage of the brain with electrodes in situ in the same type of fixative until autopsy on the following day. Tissue samples were processed for paraffin embedment and serially sectioned in the coronal plane. The following stains were used: Nissl (for neurons), H&E (general stain) and Masson's Trichrome (for connective tissue).

RESULTS

I. **Autopsy Findings.** Following electrode removal at autopsy, the electrodes were examined for abnormalities (e.g., bent tips) and stored for scanning electron microscopy.

A. Twenty-four hour implants. Irrespective of the type of electrode implant, a common finding was a small amount of patchy epidural hemorrhage. Also, for each size of electrode tip and method of insertion, 3 to 5 sites showed small amounts of

TABLE I
IC TIP CONFIGURATION vs. HEMORRHAGE (ACUTE)

IC #	TIP (μm DIA)	TYPE INSERT.	AUTOPSY	HISTOLOGY	
				MAX. LINEAR DIMENSION OF INTER-STITIAL HEMORR. (μm)	NEURONS
108(LA)	1	M	Subdural hemorr.	400	N
108(LP)	1	M	N	875	N
110(LA)	1	M	Subdural hemorr.	500	N
110(LP)	1	M	N	600	N
112(RA)	1	M	N	125	Occas. mech. & hyperchr.
112(RP)	1	M	N	None	N
114(LA)	1	S	Subdural hemorr.	550	Occas. mech.
116(LP)	1	S	N	550	N
108(RA)	3	M	Subdural hemorr.	100	N
108(RP)	3	M	Subdural hemorr.	700	Occas. mech.
110(RA)	3	M	N	300	Occas. mech.
110(RP)	3	M	N	230	N
112(LA)	3	M	N	425	Occas. mech. & hyperchr.
112(LP)	3	M	N	250	N
114(RA)	3	S	Subdural hemorr.	700	Occas. mech.
114(RP)	3	S	Subdural hemorr.	500	N
116(RA)	3	S	Subdural hemorr.	300	N
116(RP)	3	S	Subdural hemorr.	350	Occas. mech. & stellate
107(LA)	6	M	N	500	Occas. mech.
107(LP)	6	M	N	600	Occas stellate & hyperchr
109(LA)	6	M	Small subdural hem.	400	N
109(LP)	6	M	Small subdural hem.	650	N
111(RA)	6	M	N	550	Occas stellate & hyperchr
111(RP)	6	M	Small hem. @ entry	950	N
115(LA)	6	S	Subdural hemorr.	None	N
115(LP)	6	S	N	540	N
117(LA)	6	S	Subdural hemorr.	600	N
117(LP)	6	S	N	400	Occas. mech.

TABLE I
IC TIP CONFIGURATION vs. HEMORRHAGE
(ACUTE - cont.)

IC #	TIP (μm DIA)	TYPE INSERT.	AUTOPSY	HISTOLOGY	
				MAX. LINEAR DIMENSION OF INTER-STITIAL HEMORR. (μm)	NEURONS
107(RA)	Facet	M	N	500	Occas. mech.
107(RP)	Facet	M	Small hem. around entry	1,375	Occas. stellate & hyperchrom.
109(RA)	Facet	M	Small hem. around entry	100	N
109(RP)	Facet	M	Small hem. around entry	300	N
111(LA)	Facet	M	Subdural hemorr.	300	Occas. stellate & hyperchrom.
111(LP)	Facet	M	N	600	N
115(RA)	Facet	S	N	300	N
115(RP)	Facet	S	N	1,000	N
117(RA)	Facet	S	N	250	N
117(RP)	Facet	S	Punctate hem. @ entry site	600	Occas. mech.

(L) = Left (R) = Right (A) = Anterior (P) = Posterior (M) = Manual insertion
(S) = Stereotaxic insertion (N) = Normal Mech. = Mechanically flattened
Hyperchr. = Hyperchromic

TABLE I

IC TIP CONFIGURATION vs. HEMORRHAGE

(CHRONIC)

IC #	TIP (μm DIA)	TYPE INSERT.	DURATION (DAYS)	AUTOPSY	HISTOLOGY	
					HEMORR.	NEURONS
113(L-)	6	S	14	Cortex adherent to electrode	None	N
113(RA)	6	S	14	N	None	M
113(RP)	6	S	14	N	None	N
106(LA)	1	S	28	Tissue on underside of matrix	None	Occas. Mech.
106(LP)	1	S	28	Tissue on underside of matrix	None	N
106(RA)	3	S	28	Tissue on underside of matrix	None	N
106(RP)	3	S	28	Tissue on underside of matrix	None	N

(L) = Left (R) = Right (A) = Anterior (P) = Posterior (M) = Manual insertion
(S) = Stereotaxic insertion Mech. = Mechanically flattened Hyperchr. = Hyperchromic
(N) = Normal

subdural hemorrhage. In a few instances, there were small hemorrhages beneath the electrode matrices. After removal of the matrices, a punctate hemorrhage was occasionally present at the electrode entry site. A universal finding was a clearly-defined cortical depression made by the array matrix (0.2 to 0.5 mm in depth).

B. Fourteen-day implants. The single animal in this category (IC-113) showed no hemorrhage or other abnormalities at any electrode site.

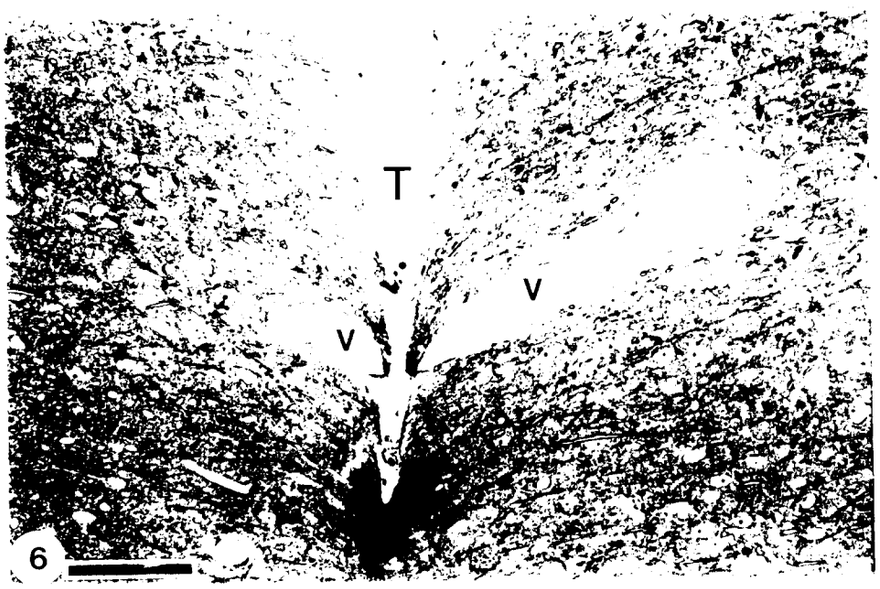
C. Twenty-eight day implant (one animal). Slight epidural hemorrhage was present at both operative sites. There was firm attachment of the underside of the dura to the matrices of all 4 electrodes which resulted in inadvertent withdrawal of all electrodes during resection of the dura. Epidural hemorrhage was not present. All matrices appeared to have a thin layer of tissue lining their inferior surfaces.

II. Histologic Findings.

A. Twenty-four hour implants. Infection was not present at any site and there was no difference between the histologic findings following manual insertion (M) vs. introduction of the electrodes with the stereotaxic frame-mounted introducer device (S). Because of the short implant duration, gliosis, microcavitations and vascular changes (hyperplasia and hypertrophy) were not present.

1. 1 μm diameter tips, 10 implants. Two of the 10 sites were excluded from the study because the electrode tips were found to be bent at autopsy. Only one site (IC-112, right hemisphere) showed no interstitial hemorrhage at any level. Fortuitously, a major hemorrhage was averted by the "near miss" of a large blood vessel at a depth of 1,375 μm (Figs. 5 & 6).

By far, the most typical finding was that of interstitial hemorrhages emanating from the electrode track and extending in one or more directions for 100 to 900 μm (Fig. 7). The origin of the hemorrhage was not obvious but probably was one of the numerous blood vessels in the path of the penetrating electrode. Aside from a few mechanically-distorted and somewhat hyperchromic neurons, the neurons near all tracks appeared normal.



Figs. 5 & 6. IC-112. Right posterior track, at the site of an electrode with a 1 µm diameter rounded tip, Implanted for 1 day. The plastic section is cut through the right posterior track (T) left by a 1 µm round-tipped electrode. This track is one of only two in the entire series of 40 one-day implant sites which did not sustain a hemorrhage. Apparently, the electrode had narrowly missed a sizable blood vessel (V). Bars = 250 µm and 100 µm, respectively. These and Figs. 8, 14 and 15 are micrographs of plastic embedded tissue. The remaining figures are micrographs from paraffin-embedded, Nissl-stained tissue. All figures are of vertically cut cortex through the implant sites.

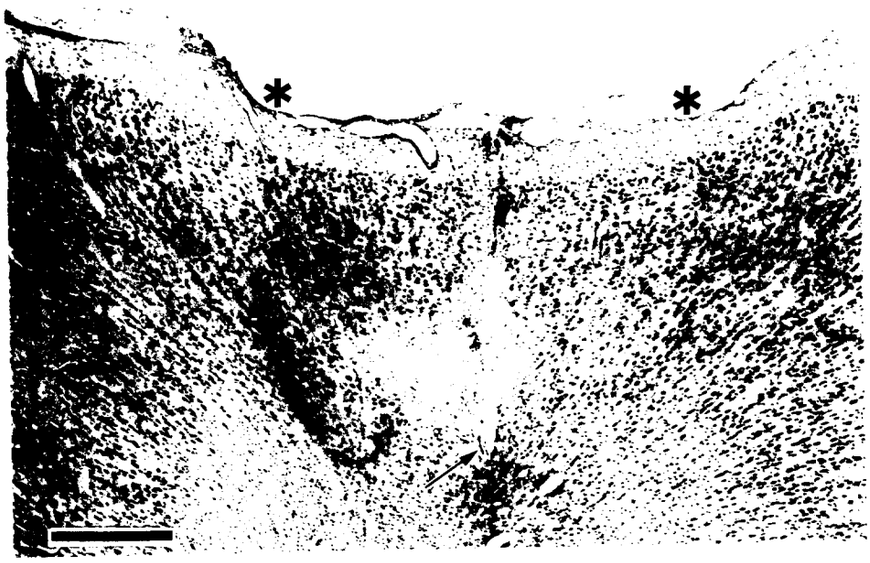


Fig. 7. IC-114. Left anterior track, at the site of an electrode with a 1 μm diameter rounded tip. The Implant duration was 1 day. The photograph was taken at low magnification, and shows the 400 μm deep depression in the surface of the cortex (*) left by the epoxy matrix. The deep part of the track (arrow) is accompanied by a hemorrhage extending about 650 μm laterally. Much neural tissue has been obliterated by the hemorrhage. The exact source of the hemorrhage was not localized in this or subsequent micrographs. Bar = 500 μm.

2. 3 μm diameter rounded tips, 10 implants. All sites showed hemorrhage extending up to 700 μm away from the track, irrespective of whether the electrodes were implanted manually or stereotaxically. The amount of mechanical distortion of neurons near the tracks was also independent of the method of insertion. This mechanical distortion was seen at 5 of the 10 implant sites. One phenomenon seen in the series and occasionally in the other series was the presence of one remarkably straight, non-hemorrhagic segment of the side of the track in contradistinction to the opposite side which showed a tortuous zone, devoid of tissue but filled with extravasated RBC (Fig. 8).

3. 6 μm diameter rounded tips, 10 implants. One implant site (IC-115, left anterior site) was free of hemorrhage and all adjacent neurons appeared normal. Hemorrhages at the 9 remaining sites varied in size and extended from 100 to 950 μm away from the track. Figs. 9 and 10 show a track in which the hemorrhage accompanied the track for 600 μm and was 100 μm in width. Numerous neutrophils had infiltrated the hemorrhagic zone. This area no longer contained neural tissue and it appeared as if the hemorrhage had disrupted and displaced the neuropil adjacent to the track. At 4 tracks, a few neurons showed either mechanical compression or were hyperchromic and stellate. For the most part, normal-appearing neurons prevailed at all sites.

4. Faceted tips, 10 implants. As noted above, 4 of the 10 implant sites showed a few neurons flattened by mechanical compression or exhibiting a stellate profile, and hyperchromism. Interstitial hemorrhage was present near all tracks irrespective of the method of implantation of the probes. Near the manually-implanted probe, the length of the hemorrhage varied from 100 to 1,375 μm while those at the site of stereotaxically-implanted probes were 250 to 1,000 μm in length. The hemorrhage originated at various depths along the track (e.g., superficially or deep segments of the track as in Figs. 11 and 12).

Fig. 8. IC-110. Midportion of right posterior track (T) at the site of an electrode with a 3 µm rounded tip. The implant duration was 1 day. One edge of the track is remarkably straight while the opposite side has been obliterated by the hemorrhage and the defect has been filled by extravasated RBC. Nearby neurons appear normal. Bar = 100 µm.

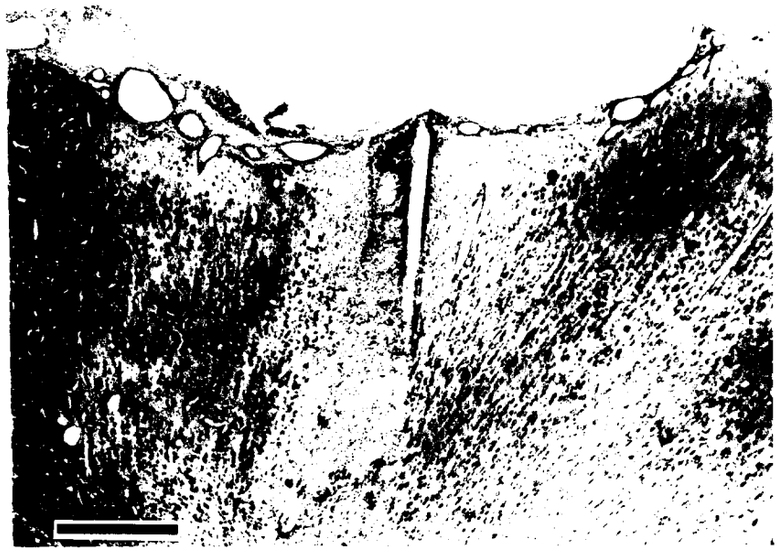


Fig. 9. IC-117. Left anterior site of an electrode with a 6 µm diameter rounded tip. The implant duration was 1 day. The 380 µm deep depression in the surface of the cortex made by the array matrix is clearly evident. The track reaches a depth of 1,500 µm. A hemorrhage lies adjacent to one entire edge of the track and the upper half of the hemorrhagic zone is infiltrated by neutrophils. An approximately 300 x 400 µm hemorrhage lies adjacent to the lower end of the track. Bar = 500 µm.

Fig. 10. Higher magnification of the track (T) and tip area seen in the previous micrograph. The arrows delineate the edge of the hemorrhagic area still packed with RBC. Numerous neutrophils have infiltrated portions of the hemorrhage (arrowheads). Adjacent neurons appear intact. Bar = 200 µm.

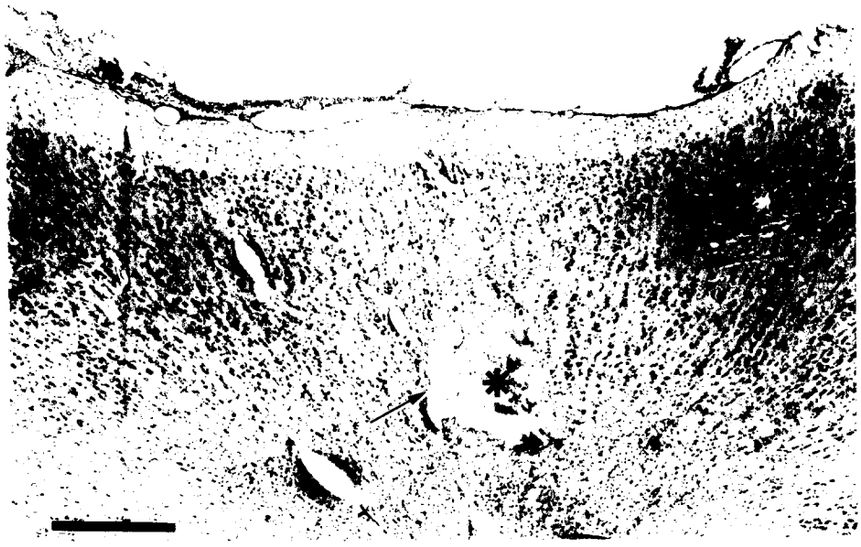


Fig. 11. IC-117. Right posterior electrode site of an electrode with a faceted tip, implanted for 1 day. The deep end of the track (arrow) is accompanied by a large, rectangular hemorrhage (asterisk). Bar = 500 μm.

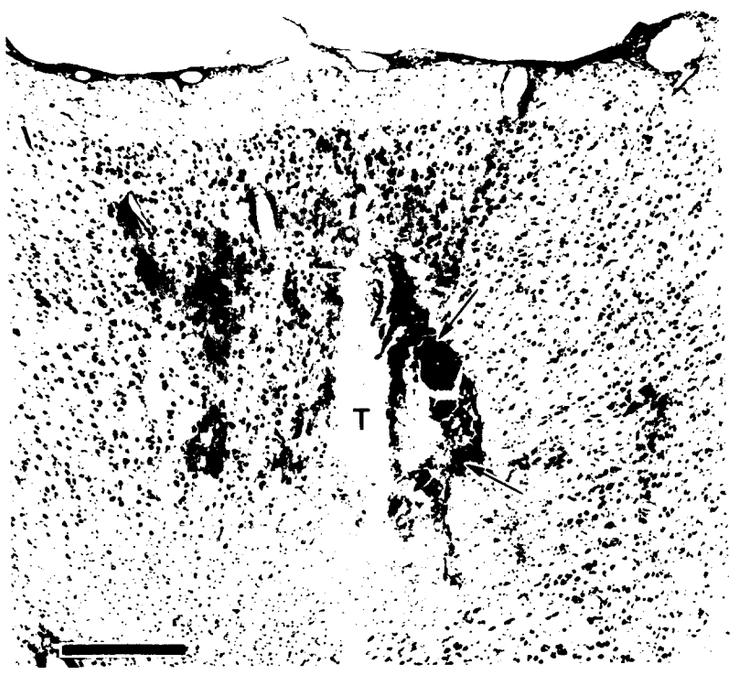


Fig. 12. IC-115. Right anterior electrode site of an electrode with a faceted tip. Implant duration = 1 day. A large hemorrhage (arrows) accompanies the entire lower half of the track (T). Apparently, some of the macerated edges of the track (caused by the hemorrhage) have been avulsed during withdrawal of the electrode at autopsy resulting in a distortion and widening of the track site. Bar = 400 μm.

B. Fourteen-day implants. A single animal (IC-113) was implanted for this duration and, of the three sites at which probes with 6 μm diameter, rounded-tips were implanted, none showed any interstitial hemorrhage and neurons near the tracks appeared normal. As in previous series where the implants had been in place for at least several days,vascular hyperplasia and hypertrophy were present at all sites. In addition, moderate gliosis accompanied the track. Perivascular cuffing with lymphocytes was present (Fig. 13).

C. Twenty-eight day implants. (One animal, 4 implants.) Two of the electrodes had a 1 μm diameter, rounded tips while the other two had 3 μm diameter rounded tips. There was virtually no difference between the findings at sites adjacent to the 2 types of electrodes. The tracks were straight-edged and lined by a thin, compact connective tissue sheath. Interstitial hemorrhage was never present, although the tissue adjacent to some portions of the tracks consisted of a zone of fibrosis suggesting the resolution of a former hemorrhage (Fig. 14). Vascular hyperplasia was a common finding. Normal-appearing neurons predominated at all sites (Figs. 14 & 15). At one site, a few neurons were mechanically-flattened.

DISCUSSION

The high incidence of interstitial hemorrhages at the site of implantation of slender microelectrode probes reflects the large numbers of blood vessels in the cortex and the high probability of the electrode tip encountering one or more vessels during penetration. For these reasons, it is not surprising that the hemorrhages occurred at all levels (depths) along the tracks. Further, neither method of electrode implantation (manual or with the frame-mounted microdrive) was superior, at least with respect to reducing the number and size of hemorrhages at the sites of electrodes implanted for 1 day. The absence of hemorrhage at the sites of electrodes implanted for longer periods (14 and 28 days) indicates that injury to the blood vessels occurs early, probably during or shortly after implantation of the probes.

In a few instances, one side of the electrode track from the 1-day implants was straight and free of hemorrhage in contradistinction to the opposite side which was disrupted and effaced, with

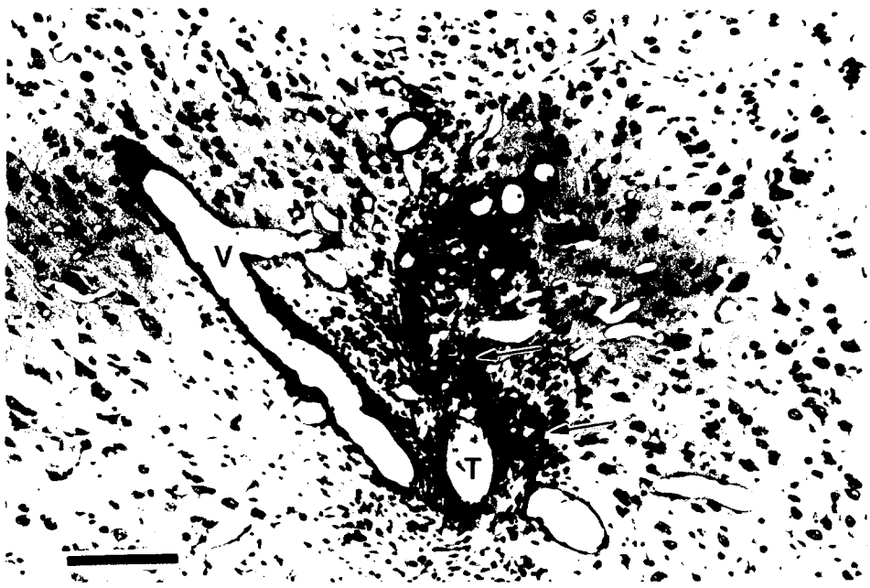


Fig. 13. IC-113. Right posterior electrode site of an electrode with a rounded tip, 6 µm in diameter.. The implant duration was 14 days. A deep segment of the track (T) is surrounded by marked gliosis (arrows). Neither cavitation nor hemorrhage is present. Vascular hypertrophy (V) and some vascular hyperplasia are present. At higher magnification, neurons in this area appeared normal. Bar = 150 µm.

Fig. 14. IC-106. Left posterior electrode site of an electrode with a 1 µm diameter, rounded tip. The duration of the implant was 28 days. The track (T) is lined by a thin connective tissue sheath. There is no evidence of cavitations or a fresh hemorrhage although the gliotic area (*) probably represents the site of an earlier hemorrhage and resolution. Vascular hyperplasia is present on both sides of the track. Though not evident in the micrograph, nearby neurons, apart from the gliotic area, appeared normal. Bar = 100 µm.

Fig. 15. IC-106. Right posterior electrode site of an electrode with a 3 µm rounded tip. The implant duration was 28 days. The site of the electrode tip (T) is 1,200 µm beneath the surface of the cortex. A cluster of glial cells (G) lies just beneath the tip. Neither hemorrhage nor cavitation is present. Nearby neurons appeared normal. Bar = 50 µm.

subsequent replacement by extravasated RBC. This phenomenon undoubtedly represents the "grazing" puncture of a vessel by the electrode, thus producing a unilateral accumulation of blood along the track. Penetration of a blood vessel closer to its center would probably cause hemorrhage along the entire periphery of the track.

Serial sections of the track of one of only two non-hemorrhagic sites in the entire series of 40 one-day implants, showed the "near miss" of a large blood vessel by the electrode. This finding supports the concept that avoiding contact with cortical blood vessels is virtually impossible and successful implantations must depend on minimizing the injury to the vessels when they are encountered by the probe's tip. A slow penetration, of several minutes duration, might be expected to "shoulder aside" the blood vessel and thus avert vascular rupture.

Of no small consequence is the rupture and extravasation of blood adjacent to the track inasmuch as this often leads to destruction of a significant amount of neuropil and a significant number of neurons. The latter constitute the "effector organ" for electrical stimulation, and, in fact, constitute the very basis for use of microelectrodes. Work currently underway is focusing on the effects of very rapid and very slow electrode insertion which hopefully will result in a reduction in vascular injury.

REFERENCES

Boast, C.A., Reid, S.A., Johnson, P.N. and Zornetzer, S.F.: A caution to brain scientists: Unexpected hemorrhagic vascular damage resulting from mere electrode implantation. Brain Res., 103:527-534, 1976.

WORK NEXT QUARTER

We will continue these studies by examining the effects of slow and fast insertion of microelectodes into the cerebra cortex. These probes will have tip configurations similar to those described in this report. This series will include longer implant durations than those of the present series. It is anticipated that slow insertion of the probes will decrease the shearing force exerted on blood vessels and adjacent tissue. Slow insertion of microelectrodes with beveled tips might easily penetrate the pia while subjecting the brain parenchyma to minimal stretching and shearing. On the other hand, a rounded tip introduced rapidly through the pia, followed by slow insertion to its final depth might be expected to minimize pial dimpling while also "nudging aside" the underlying blood vessels, and therby averting vascular rupture.